AMENDMENTS

Amendments to the Claims

1-3. (Canceled)

4. (Previously presented) A botulinum toxin serotype A (BoNT/A) substrate, comprising:

(a) a donor fluorophore;

(b) an acceptor fluorophore having an absorbance spectrum overlapping the emission spectrum of said donor fluorophore; and

(c) a BoNT/A recognition sequence comprising a cleavage site, wherein said cleavage site intervenes between said donor fluorophore and said acceptor fluorophore;

wherein, under the appropriate conditions, resonance energy transfer is exhibited between said donor fluorophore and said acceptor fluorophore.

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5. (Currently amended) The BoNT/A substrate of claim 4, wherein said BoNT/A substrate comprises at least six consecutive residues of SNAP-25, or a peptidomimetic thereof, said six consecutive residues comprising Gln-Arg, or a peptidomimetic thereof.

6. (Currently amended) The BoNT/A substrate of claim 5, wherein said BoNT/A substrate comprises at least six consecutive residues of a human SNAP-25, or a peptidomimetic thereof, said six consecutive residues comprising Gln_{197}-Arg_{198}, or a peptidomimetic thereof.

7. (Previously presented) The BoNT/A substrate of claim 6, wherein said BoNT/A recognition sequence comprises SEQ ID NO: 27, or a peptidomimetic thereof.

8. (Previously presented) The BoNT/A substrate of claim 6, wherein said BoNT/A recognition sequence comprises SEQ ID NO: 29, or a peptidomimetic thereof.

9-44. (Canceled)

45. (Previously presented) The BoNT/A substrate of claim 4, wherein said substrate can be cleaved with an activity of at least 1 nanomole/minute/milligram toxin.

46. (Previously presented) The BoNT/A substrate of claim 4, wherein said BoNT/A substrate can be cleaved with an activity of at least 20 nanomole/minute/milligram toxin.

47. (Previously presented) The BoNT/A substrate of claim 4, wherein said BoNT/A substrate can be cleaved with an activity of at least 50 nanomole/minute/milligram toxin.

48. (Previously presented) The BoNT/A substrate of claim 4, wherein said BoNT/A substrate can be cleaved with an activity of at least 100 nanomole/minute/milligram toxin.

49. (Previously presented) The BoNT/A substrate of claim 4, wherein said BoNT/A substrate can be cleaved with an activity of at least 150 nanomole/minute/milligram toxin.

50. (Canceled)

51. (Previously presented) The BoNT/A substrate of claim 4, wherein said acceptor fluorophore has a fluorescent lifetime of at least 1 microsecond.

52. (Previously presented) The BoNT/A substrate of claim 4, wherein said donor fluorophore is BODIPY®–530/550 (4,4-difluoro-5,7-diphenyl-4-bora-3a,4a-diaza-S-indacene).

53. (Previously presented) The BoNT/A substrate of claim 4, wherein said donor fluorophore is fluorescein.

54. (Canceled)

55. (Previously presented) The BoNT/A substrate of claim 4, wherein said donor fluorophore has an emissions maxima of about 603 nm.

56. (Canceled)

57. (Previously presented) The BoNT/A substrate of claim 4 or claim 53, wherein said acceptor fluorophore is tetramethylrhodamine.

58. (Previously presented) The BoNT/A substrate of claim 4 or claim 55, wherein said acceptor fluorophore has an excitation maxima of about 679 nm.

59. (Previously presented) The BoNT/A substrate of claim 4 or claim 52, wherein said acceptor fluorophore is BODIPY®–542/563 (4,4 difluoro-5-p-methoxyphenyl-4-bora-3a,4a-diaza-S-indacene).

60. (Previously presented) The BoNT/A substrate of claim 4, wherein said donor fluorophore is BODIPY®–542/563 (4,4 difluoro-5-p-methoxyphenyl-4-bora-3a,4a-diaza-S-indacene).

61. (Previously presented) The BoNT/A substrate of claim 4 or claim 60, wherein said acceptor fluorophore is BODIPY®–564/570 (4,4 difluoro-5-styryl-4-bora-3a,4a-diaza-S-indacene).

62. (Previously presented) The BoNT/A substrate of claim 4, wherein said donor fluorophore is Cy3.

63. (Previously presented) The BoNT/A substrate of claim 4 or claim 62, wherein said acceptor fluorophore is Cy5.

64-95. (Canceled)

96. (Previously presented) The BoNT/A substrate of claim 4, wherein said BoNT/A substrate has at most 20 residues.

97. (Previously presented) The BoNT/A substrate of claim 4, wherein said BoNT/A substrate has at most 40 residues.

98. (Previously presented) The BoNT/A substrate of claim 4, wherein said BoNT/A substrate has at most 50 residues.

99. (Previously presented) The BoNT/A substrate of claim 4, wherein said BoNT/A substrate has at most 100 residues.

100. (Previously presented) The BoNT/A substrate of claim 4, wherein said BoNT/A substrate has at most 150 residues.

101. (Previously presented) The BoNT/A substrate of claim 4, wherein said BoNT/A substrate has at most 200 residues.

102. (Previously presented) A botulinum toxin serotype A (BoNT/A) substrate, comprising:

(a) a donor fluorophore;

(b) an acceptor having an absorbance spectrum overlapping the emission spectrum of said donor fluorophore; and

(c) a BoNT/A recognition sequence comprising a cleavage site, said BoNT/A recognition sequence comprising the amino acids 191 to 202 of SEQ ID NO: 2, or a peptidomimetic thereof;

wherein said cleavage site intervenes between said donor fluorophore and said acceptor;

wherein said donor fluorophore, said acceptor, or both said donor fluorophore and said acceptor is not positioned within amino acids 191 to 202 of SEQ ID NO: 2, or a peptidomimetic thereof; and

wherein, under the appropriate conditions, resonance energy transfer is exhibited between said donor fluorophore and said acceptor.

103. (Previously presented) The BoNT/A substrate of claim 102, wherein said BoNT/A recognition sequence comprises SEQ ID NO: 29, or a peptidomimetic thereof.

104. (Previously presented) The substrate of claim 102, wherein said BoNT/A recognition sequence comprises SEQ ID NO: 30, or a peptidomimetic thereof.

105. (Previously presented) The BoNT/A substrate of any of claims 102, 103 or 104, wherein said BoNT/A substrate can be cleaved with an activity of at least 1 nanomole/minute/milligram toxin.

106. (Previously presented) The BoNT/A substrate of any of claims 102, 103 or 104, wherein said BoNT/A substrate can be cleaved with an activity of at least 20 nanomole/minute/milligram toxin.

107. (Previously presented) The BoNT/A substrate of any of claims 102, 103 or 104, wherein said BoNT/A substrate can be cleaved with an activity of at least 50 nanomole/minute/milligram toxin.

108. (Previously presented) The BoNT/A substrate of any of claims 102, 103 or 104, wherein said BoNT/A substrate can be cleaved with an activity of at least 100 nanomole/minute/milligram toxin.

109. (Previously presented) The BoNT/A substrate of any of claims 102, 103 or 104, wherein said BoNT/A substrate can be cleaved with an activity of at least 150 nanomole/minute/milligram toxin.

110. (Previously presented) The BoNT/A substrate of claim 102, wherein said acceptor is an acceptor fluorophore.

111. (Previously presented) The BoNT/A substrate of claim 110, wherein said acceptor fluorophore has a fluorescent lifetime of at least 1 microsecond.

112. (Previously presented) The BoNT/A substrate of claim 102, wherein said acceptor is a non-fluorescent acceptor.

113. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore is fluorescein.

114. (Currently amended) The BoNT/A substrate of claim 102, wherein said donor fluorophore is EDANS.

115. (Previously presented) The BoNT/A substrate of claim 102 or 113, wherein said acceptor is a fluorophore, said acceptor fluorophore being tetramethylrhodamine.

116. (Currently amended) The BoNT/A substrate of claim 102 or 114, wherein said acceptor is a non-fluorescent acceptor, said non-fluorescent acceptor being ~~DANCYL~~ DABCYL.

117. (Currently amended) The BoNT/A substrate of claim 112, wherein said non-fluorescent acceptor is DNP, DABCYL, or DABSYL ~~or QSY® 7~~.

118. (Previously presented) The BoNT/A substrate of claim 102, wherein said BoNT/A substrate has at most 100 residues.

119. (Previously presented) The BoNT/A substrate of claim 102, wherein said BoNT/A substrate has at most 50 residues.

120. (Previously presented) The BoNT/A substrate of claim 102, wherein said BoNT/A substrate has at most 40 residues.

121. (Previously presented) The BoNT/A substrate of claim 102, wherein said BoNT/A substrate has at most 20 residues.

122. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most fifteen residues.

123-125. (Canceled)

126. (Previously presented) A botulinum toxin serotype A (BoNT/A) substrate, comprising:

(a) a donor fluorophore;

(b) an acceptor having an absorbance spectrum overlapping the emission spectrum of said donor fluorophore; and

(c) a BoNT/A recognition sequence comprising a cleavage site, said BoNT/A recognition sequence comprising SEQ ID NO: 29, or a peptidomimetic thereof;

wherein said cleavage site intervenes between said donor fluorophore and said acceptor;

wherein said donor fluorophore or said acceptor is genetically encoded; and

wherein, under the appropriate conditions, resonance energy transfer is exhibited between said donor fluorophore and said acceptor.

127. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore is genetically encoded.

128. (Previously presented) The BoNT/A substrate of claim 126, wherein said acceptor is genetically encoded.

129. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore and said acceptor are genetically encoded.

130. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A substrate comprises at least six consecutive residues of SNAP-25, said six consecutive residues comprising Gln-Arg.

131. (Previously presented) The BoNT/A substrate of claim 130, wherein said BoNT/A substrate comprises at least six consecutive residues of a human SNAP-25, said six consecutive residues comprising Gln_{197}-Arg_{198}.

132. (Previously presented) The BoNT/A substrate of claim 131, wherein said BoNT/A substrate comprises the amino acid sequence Glu-Ala-Asn-Gln-Arg-Ala-Thr-Lys (SEQ ID NO: 1).

133. (Previously presented) The BoNT/A substrate of claim 131, wherein said BoNT/A recognition sequence comprises SEQ ID NO: 27.

134. (Previously presented) The BoNT/A substrate of either claim 126 or claim 129, wherein said BoNT/A substrate can be cleaved with an activity of at least 1 nanomole/minute/milligram toxin.

135. (Previously presented) The BoNT/A substrate of either claim 126 or claim 129 wherein said BoNT/A substrate can be cleaved with an activity of at least 20 nanomole/minute/milligram toxin.

136. (Previously presented) The BoNT/A substrate of either claim 126 or claim 129, wherein said BoNT/A substrate can be cleaved with an activity of at least 50 nanomole/minute/milligram toxin.

137. (Previously presented) The BoNT/A substrate of either claim 126 or claim 129, wherein said BoNT/A substrate can be cleaved with an activity of at least 100 nanomole/minute/milligram toxin.

138. (Previously presented) The BoNT/A substrate of either claim 126 or claim 129, wherein said BoNT/A substrate can be cleaved with an activity of at least 150 nanomole/minute/milligram toxin.

139. (Previously presented) The BoNT/A substrate of claim 126, wherein said acceptor is an acceptor fluorophore.

140. (Previously presented) The BoNT/A substrate of claim 139, wherein said acceptor fluorophore has a fluorescent lifetime of at least 1 microsecond.

141. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A substrate has at most 400 residues.

142. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A substrate has at most 500 residues.

143. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A substrate has at most 600 residues.

144. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A substrate has at most 700 residues.

145. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at most fifteen residues.

146. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at most thirty-five residues.

147-148. (Canceled)

149. (Previously presented) The BoNT/A substrate of claim 4, wherein said donor fluorophore and said acceptor are separated by at most ten residues.

150. (Previously presented) The BoNT/A substrate of claim 4, wherein said donor fluorophore and said acceptor are separated by at most fifteen residues.

151. (Previously presented) The BoNT/A substrate of claim 4, wherein said donor fluorophore and said acceptor are separated by at most twenty residues.

152. (Previously presented) The BoNT/A substrate of claim 4, wherein said donor fluorophore and said acceptor are separated by at most thirty residues.

153. (Previously presented) The BoNT/A substrate of claim 4, wherein said donor fluorophore and said acceptor are separated by at most forty residues.

154. (Previously presented) The BoNT/A substrate of claim 4, wherein said BoNT/A substrate is selected from the group consisting of SEQ ID NO: 85, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94 and SEQ ID NO: 95.

155. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most twenty residues.

156. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most twenty-five residues.

157. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most thirty residues.

158. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most thirty-five residues.

159. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most forty residues.

160. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A substrate has at least 300 residues.

161. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A substrate has at least 400 residues.

162. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A substrate has at least 500 residues.

163. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A substrate has at least 600 residues.

164. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A substrate has at least 700 residues.

165. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at most twenty residues.

166. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at most twenty-five residues.

167. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at most thirty residues.

168. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at most forty residues.

169. (Canceled).

170. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at least 50 residues.

171. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at least 75 residues.

172. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at least 100 residues.

173. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at least 125 residues.

174. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at least 150 residues.

175. (Previously presented) The BoNT/A substrate of claim 126, wherein said donor fluorophore and said acceptor are separated by at least 200 residues.

176. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most ten residues.

177. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most eight residues.

178. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore and said acceptor are separated by at most six residues.

179. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore is not positioned within amino acids 191 to 202 of SEQ ID NO: 2, or a peptidomimetic thereof.

180. (Previously presented) The BoNT/A substrate of claim 102, wherein said acceptor is not positioned within amino acids 191 to 202 of SEQ ID NO: 2, or a peptidomimetic thereof.

181. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore and said acceptor are not positioned within amino acids 191 to 202 of SEQ ID NO: 2, or a peptidomimetic thereof.

182. (Previously presented) The BoNT/A substrate of claim 102, wherein said BoNT/A substrate comprises at least six consecutive residues of SNAP-25, said six consecutive residues comprising Gln-Arg.

183. (Previously presented) The BoNT/A substrate of claim 182, wherein said BoNT/A substrate comprises at least six consecutive residues of a human SNAP-25, said six consecutive residues comprising Gln_{197}-Arg_{198}.

184. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore is BODIPY®–530/550 (4,4 difluoro 5,7 diphenyl 4 bora-3a,4a-diaza-S-indacene).

185. (Previously presented) The BoNT/A substrate of claim 102 or claim 184, wherein said acceptor is a fluorophore, said acceptor fluorophore being BODIPY®–542/563 (4,4 difluoro-5-p-methoxyphenyl-4-bora-3a,4a-diaza-S-indacene).

186. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore is BODIPY®–542/563 (4,4 difluoro-5-p-methoxyphenyl-4-bora-3a,4a-diaza-S-indacene).

187. (Previously presented) The BoNT/A substrate of claim 102 or claim 186, wherein said acceptor is a fluorophore, said acceptor fluorophore being BODIPY®–564/570 (4,4 difluoro-5-styryl-4-bora-3a,4a-diaza-S-indacene).

188. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore is Cy3.

189. (Previously presented) The BoNT/A substrate of claim 102 or claim 188, wherein said acceptor is a fluorophore, said acceptor fluorophore being Cy5.

190. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore has an emission maxima of about 603 nm.

191. (Previously presented) The BoNT/A substrate of claim 102 or claim 190, wherein said acceptor is a fluorophore, said acceptor fluorophore having an excitation maxima having an excitation maxima of about 679 nm.

192. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore has an emission maxima of about 690 nm.

193. (Previously presented) The BoNT/A substrate of claim 102 or claim 192, wherein said acceptor is a fluorophore, said acceptor fluorophore having an excitation maxima of about 749 nm.

194. (Previously presented) The BoNT/A substrate of claim 102, wherein said donor fluorophore is pyrene.

195. (Previously presented) The BoNT/A substrate of claim 102 or claim 194, wherein said acceptor is a fluorophore, said acceptor fluorophore being coumarin.

196. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A substrate comprises at most 300 residues.

197. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A substrate comprises at most 350 residues.

198. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A recognition sequence comprises amino acids 137 to 206 of SEQ ID NO: 2.

199. (Previously presented) The BoNT/A substrate of claim 126, wherein said BoNT/A recognition sequence comprises amino acids 134 to 206 of SEQ ID NO: 2.

200. (Previously presented) The BoNT/A substrate of either claim 127 or 129, wherein said genetically encoded donor fluorophore is selected from the group consisting of a blue fluorescent protein, a cyan fluorescent protein, a green fluorescent protein, a yellow fluorescent protein and a red fluorescent protein.

201. (Previously presented) The BoNT/A substrate of either claim 128 or 129, wherein said genetically encoded acceptor is a fluorophore, said genetically encoded acceptor fluorophore selected from the group consisting of a blue fluorescent protein, a cyan fluorescent protein, a green fluorescent protein, a yellow fluorescent protein and a red fluorescent protein.

202. (Previously presented) The BoNT/A substrate of claim 129, wherein said genetically-encoded acceptor is a fluorophore.

203. (Previously presented) The BoNT/A substrate of claim 196, wherein said donor fluorophore is a blue fluorescent protein, said acceptor fluorophore is a green fluorescent protein and said BoNT/A recognition sequence comprises SEQ ID NO: 29.

204. (Previously presented) The BoNT/A substrate of claim 196, wherein said donor fluorophore is a blue fluorescent protein, said acceptor fluorophore is a green fluorescent protein and said BoNT/A recognition sequence comprises amino acids 137-206 of SEQ ID NO: 2.

205. (Previously presented) The BoNT/A substrate of claim 196, wherein said donor fluorophore is a blue fluorescent protein, said acceptor fluorophore is a green fluorescent protein and said BoNT/A recognition sequence comprises amino acids 134-206 of SEQ ID NO: 2.

206. (Previously presented) The BoNT/A substrate of claim 196, wherein said donor fluorophore is a blue fluorescent protein, said acceptor fluorophore is a green fluorescent protein and said BoNT/A recognition sequence comprises SEQ ID NO: 2.

207. (Previously presented) The BoNT/A substrate of claim 196, wherein said donor fluorophore is a green fluorescent protein, said acceptor fluorophore is a red fluorescent protein and said BoNT/A recognition sequence comprises SEQ ID NO: 29.

208. (Previously presented) The BoNT/A substrate of claim 196, wherein said donor fluorophore is a cyan fluorescent protein, said acceptor fluorophore is a yellow fluorescent protein and said BoNT/A recognition sequence comprises SEQ ID NO: 29.

209. (Previously presented) The BoNT/A substrate of claim 127, wherein said acceptor is a fluorophore.

210. (Previously presented) The BoNT/A substrate of claim 209, wherein said donor fluorophore is a green fluorescent protein, said acceptor fluorophore has an excitation

maxima of about 556 nm and said BoNT/A recognition sequence comprises SEQ ID NO: 29.

211. (Previously presented) The BoNT/A substrate of claim 209, wherein said donor fluorophore is a red fluorescent protein, said acceptor fluorophore has an excitation maxima of about 632 nm and said BoNT/A recognition sequence comprises SEQ ID NO: 29.